Arguments

REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 2, 4-20 and 46 are pending. Claims 4, 7, 9, 11 have been amended to more clearly define the present invention, and should not be construed as the surrender of any subject matter. Applicants reserve the right to file one or more continuing applications on any canceled subject matter. The amended claims have support in the original claims and specification as filed.

This amendment changes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

Rejections under 35 U.S.C. § 112, first paragraph

Claim 20

Claim 20 is rejected by the Examiner as allegedly only being enabled for the *in vitro* administration of the AAV Rep78 mutant for replication studies and it not enabled for *in vivo* therapeutic uses. The Examiner states that he has considered Dr. Hermonat's declaration and characterizes it as providing evidence of a correlation between infection with wild-type adeno-associated virus and a reduced incidence of cervical cancer in the general population. However, the examiner alleges that it does not evidence that a mutated AAV vector can be administered *in vivo* so that it is therapeutically effective for treating cancer or other papillomavirus (PV) associated diseases. The Examiner considers that the therapeutic efficacy of viral vectors administered *in vivo* is unpredictable, and that the specification needs to provide detailed teachings of how to make and administer the claimed viral vectors to treat PV disease or cancer.

Applicant respectfully disagrees with the Examiner's position and maintains that claim 20 is enabled by the specification so that a person skilled in the art could practice the present invention without undue experimentation. Applicants submit that the present

specification provides sufficient disclosure to support claim 20 in combination with what is known in the prior art. Firstly, there have been AAV gene therapy trials that show that AAV can be administered *in vivo*. For example, several press releases from Avigen in 1999 (Attachment 1) disclose the success of delivering the gene for Factor IX using an AAV based on work preformed at Children's Hospital of Philadelphia and Stanford University Medical Center that showed indications of therapeutic benefit. This same study is reported by an abstract in March 2000, by these research groups reporting success in using gene therapy to treat hemophilia B in 3 patients by delivering the gene encoding Factor IX with AAV. Additionally, other Avigen press releases disclose successfully delivering the gene encoding Factor IX using AAV to dogs (Attachment 2). Additionally, Avigen scientists showed that a single administration of AAV carrying the gene for Factor VIII produced physiological levels of biologically active Factor VIII in the plasma of animals and showed that significant levels persisted in these animals for nearly two years (Attachment 3). Another group from Avigen and Berkeley National Laboratory presented research results that dopamine activity could be restored in a primate model of Parkinson's disease following treatment with AAV carrying the gene encoding dopamine (Attachment 4). As can be seen by these disclosures of successful use of AAV to introduce genes into animals and humans, applicant submits that therapeutic efficacy of AAV is predictable to the skilled person. Applicants also refer the Examiner to U.S. patents, such as 5,670,488, disclosing AAV for gene therapy uses, such as cystic fibrosis.

The claimed AAVRep78 mutant which is administered in claim 20 binds to at least one DNA sequence obtained from a papillomavirus and the mutant's DNA binding is enhanced as compared to the binding of the corresponding wild-type AAVRep78 protein as set forth in SEQ ID NO:6 to the DNA sequence. Thus, this claim focuses on the AAVRep78 mutants that bind to papillomavirus DNA better than the AAVRep78 wild-type. These mutants are considered to be a Rep78 mutant only by the substitution of one or more amino acids for the amino acids in the wild-type Rep78. Furthermore, these mutants possess the major attributes of the full length AAV genome (FLAG) Rep78 by virtue of their ability to replicate as the wild-type AAV that contains a wild-type Rep78 gene. See for example, Figure 11 as described on pages 26, lines 10-18 of the specification, which shows the

replication of AAV Rep78 192^{HG}. In Figure 14, it is shown that the AAVRep78 192^{HG} mutant with the one amino acid change is able to more strongly inhibit HPV-induced oncogenic transformation and HPV-16 p97 promoter activity than wild-type AAV (containing a wild-type Rep78). Therefore, AAVRep78 192^{HG} has an altered biochemistry as compared to wild type AAVRep78 by being able to bind more strongly to DNA (see Figure 12) and inhibit promoter activity (AAV p5) more strongly (see Figures 10). Thus, Applicant submits that the AAVRep78 mutant is a replicating AAV as is the wild-type AAV but with enhanced inhibitory activity against HPV. This is so, particularly in view of the data presented in Figure 14 of the present invention, which uses the focus formation test, which is widely accepted as a measurement of and a model for oncogenic potential *in vivo*.

Applicants submit that treatment with AAV is predictable as supported by the known use of AAV as a vector in gene therapy as supported by the attachments discussed above. Additionally, the previously submitted declaration by Dr. Hermonat and the supporting Coker *et al.* publication showed that AAV containing the wild-type Rep78 protein inhibits papillomaviruses. As noted in the previous response, the Coker *et al.* publication disclosed in the "INTRODUCTION" that AAV is able to inhibit papillomavirus oncogene expression, papillomavirus-mediated transformation and papillomavirus replication. As commented on in the last sentence on page 83, second column of Coker *et al.*, these inhibitory effects have been mapped to the AAV Rep78 protein. Further, other publications in support of the usefulness of AAV Rep78 were provided previously. Dr. Hermonat's declaration also provides additional publications that are cited in Coker *et al.*

Additionally, Dr. Hermonat comments that the *in vitro* data presented in the present application supports the disclosed novel AAVRep78 mutants, such as the AAVRep-192^{HG}, which bind more strongly to specific DNAs as compared to the wild-type AAVRep78 protein. These stronger DNA binders are identified as useful for treating cancer. See the specification, page 26, lines 19-22. Additionally, applicants provide a publication by Storey *et al.* (Attachment 5) that shows in Table 1 that HPV-16 and HPV-18 DNA cause focus formation using baby rat kidney cells while HPV-6 and HPV-11 DNA do not. These results support a direct correlation with what occurs *in vivo* where HPV-16 and -18 have been identified as associated with cancer and HPV-6 and -11 have not. The focus formation is widely accepted

as a measurement of and a model for oncogenic potential *in vivo*. Dr. Storey comments at the end of the first column on page 1819 that

> "[t]here is a striking parallel between the *in vivo* malignancy of HPV-associated lesions and the activity of the corresponding HPV type in our co-transformation assay."

Additionally, a publication by Tsunokawa *et al.* discloses that HPV-16 DNA induced malignant transformation of NIH 3T3 cells (See attachment 6).

Therefore, in view of all of the information, publications and arguments provided above, applicant submits that claim 20 is enabled, and as a result, it is requested that this rejection be withdrawn.

Claims 2, 4-20 and 46

Claims 2, 4-20 and 46 are rejected because the Examiner alleges that the specification, while being enabling for an AAVRep78 mutant which demonstrates enhanced binding to HPV16 and decreasing binding to itself, is not enabled for increased or decreased binding to HIV and oncogenes as compared with wild type. The Examiner states that the specification does not demonstrate either decreased or enhanced binding to either HIV or to an oncogene. Applicant disagrees with the Examiner's position and directs him to page 5, beginning at line 5, where the many proteins that AAVRep78 regulates are disclosed as follows:

> AAV Rep78 regulates a variety of heterologous genes. C-H-*ras* (Katz, *et al.*, *Cancer Research* 46:3023-3026 (1986); Hermonat, P.L., *Cancer Research* 51:3373-3377 (1991); Khleif, *et al.*, *Virology* 181:738-741 (1991)), c-*fos* (Klein-Bauernschmitt, *et al.*, *J. Virol.* 66:419-4200 (1992); Hermonat, P.L., *Cancer Letters* 81:129-136 (1994)), c-*myc* (Klein-Bauernschmitt, *et al.*, *J. Virol.* 66:419-4200 (1992); Hermonat, P.L., *Cancer Letters* 81:129-136 (1994)), and the HIV long terminal repeat (HIV-LTR) (Rittner, *et al.*, *J. Gen. Virol.* 73:2977-2981 (1992); Antoni, *et al.*, *J. Virol.* 64:396-404 (1991)) are down-regulated by AAV Rep78, while the c-*sis* promoter is up-regulated (Wonderling, *et al.*, *J. Virol.* 70:4783-4786 (1996)). Still other genes are not affected, such as the murine osteosarcoma virus long terminal repeat (MSV-LTR)(Hermonat, P.L., *Cancer Research* 51:3373-3377 (1991)) and

> the human β-actin promoter (Horer, *et al.*, *J. Virol.* 69:5485-5496 (1995)). The largest of 4 products encoded by the AAV *rep* open reading frame (Mendleson, *et al.*, *J. Virol.* 60:823-832 (1986)), AAV Rep78, is required for AAV DNA replication (Hermonat, *et al.*, *J. Virol.* 51:329-333 (1984); Tratschin, *et al.*, *J. Virol.* 51:611-619 (1994)) and for AAV gene regulation (Labow, *et al.*, *J. Virol.* 60:251-258 (1986); Tratschin, *et al.*, *Mol. Cell. Biol.* 5:3251-3260 (1986)). AAV Rep78 carries out a range of biochemical activities which are necessary for its biological phenotypes (Im, *et al.*, *Cell* 61:447-57 (1990); Ni, *et al.*, *J. Virol.* 68:1128-1138 (1994)), including binding to promoter DNA (McCarty, *et al.*, *J. Virol.* 74:4988-4997 (1994); Batchu, *et al.*, *Cancer Letters* 86:23-31 (1994); Wonderling, *et al.*, *J. Virol.* 71:2528-2534 (1996)), and to a variety of cellular proteins (Hermonat, *et al.*, *Biochem. and Molec. Biol. Internat.* 403:409-420 (1997)), including the transcription factors Sp1 (Hermonat, *et al. Cancer Research* 56:5299-5304 (1996); Pereira, *et al.*, *J. Virol.* 71:1747-1756 (1997)), TBP (Hermonat, *et al.*, *Virology* 245:120-127 (1998)), and itself (Weitzman, *et al.*, *J. Virol.* 70:2440-2448 (1996), Hermonat, *et al.*, *FEBS Letters* 401:180-184 (1997), Smith, *et al.*, *J. Virol.* 71:4461-4471 (1997)).

Applicants provide the Batchu and Hermonat publication, (FEBS Letter, Vol. 367) (Attachment 7) cited in the present specification as disclosing the inhibitory effect of AAV Rep78 on the HIVI long terminal repeat sequence (LTR). Additionally, Batchu, *et al.*, *Cancer Letters* 86:23-31 (1994)(Attachment 8), also cited in the specification, discloses that AAVRep78 inhibits the oncogene, *ras*. Additionally, AAVRep78 inhibits other oncogenes, such as *myc*, *fos*, and *jun*. The specification as filed discloses many publications that show the effect of the binding of AAVRep78 to the many genes disclosed in the specification. Further, the present specification provides an assay to determine the extent of binding of AAVRep78 to DNA fragments. The specification also provides the entire sequence of the complete AAV-2 genome and Figure 16 provides the amino acid sequence of the wild-type AAVRep78 amino acid sequence. A person skilled in the art can mutate one or more of any one of the amino acid sequences of this disclosed sequence to create mutants as disclosed beginning on page 19 of the present specification. These AAVRep78 mutants that are prepared can then be tested in the disclosed assay for the binding to specifically identified heterologous genes. Nothing more than trial and error experimentation is required to prepare

and test these AAVRep78 mutants. With applicant's specification in hand, undue experimentation is not required to carry out the present application to its full scope. Therefore, applicant respectfully disagrees with the Examiner because there is sufficient guidance for a person skilled in the art to practice the present invention using the disclosure of the specification. In view of these supported arguments, applicant requests withdrawal of the present rejection of claims 2, 4-20 and 46.

Claim 11

Claim 11 remains rejected as not complying with the written description requirement. Claim 11 is directed to AAV Rep-77^{LG} and AAV Rep-79^{FA}. The Examiner has dismissed applicant's arguments and has interpreted them as somehow supporting the Examiner's position that the reproduction of the identical virus is unpredictable. The Examiner again asks for evidence of public availability of the starting material.

Applicant again respectfully disagrees with the Examiner's request that a deposit is necessary. Applicant has provided the complete nucleic acid for the AAV2 (Figure 15) and the nucleic acid encoding the amino acid sequence of the AAVRep78 wild-type protein (Figures 16). Applicant reiterates all of the arguments presented in the previous response and again states that the DNA sequence of the entire AAV2 is disclosed in the present application. No deposit is necessary. Additionally, the same sequence is disclosed in Srivastava *et al.* which is disclosed in the present application and is present in the National Library of Medicine NCBI Sequence site as evidenced by the attached sequence (Attachment 9). If applicant had not provided the sequence of the AAV2 genome, then the Examiner could request whether the present AAV had been deposited but as the sequence is disclosed in the present application, known and published in a scientific paper and available through on-line sequence searches, applicant submits that there is no need to provide evidence of public availability.

To reiterate the present and previous arguments, all of the AAV Rep78 mutants can be prepared from the wild-type AAV Rep78 protein as disclosed in the present application. Figure 15A-C discloses the nucleotide sequence encoding AAV Rep78 as nucleotides 321-

2186 and Figure 16 provides the corresponding amino acid sequence of the AAV Rep78 protein. Thus, the specification provides the sequences needed to prepare the AAV Rep78 mutants. The specification discloses the preparation of these mutants using known methods. Applicant submits that knowing the AAV Rep78 protein nucleic acid and amino acid sequences, a person skilled in the art can follow the specification using standard methods to modify a known amino acid sequence/nucleotide sequence, to prepare the mutants with the desired modifications from the wild-type AAV Rep78 nucleic acid and amino acid sequences, such as a single amino acid change. See the specification on page 19, lines 15 to page 20, line 13.

Applicant submits that the identical virus can be prepared by using the disclosed DNA sequence to do so. Additionally, the amino acid and nucleic acid sequences of AAV Rep 78, methods of making the AAV Rep mutants and methods for assaying for their binding is disclosed in the present application and it would not require undue experimentation to make other AAV Rep 78 modified proteins using the disclosure in the specification.

As argued above, the amino acid and nucleic acid sequences are known and provided in the specification, figures and Sequence Listing. Plasmids containing a specific AAV Rep 78 mutant can be prepared as disclosed. All of the manipulations to create other mutant proteins from known sequences are well within the skill of the artisan. Thus, a person of skill in the art would be able to prepare the claimed AAV Rep 78 mutant using the guidance in the specification for making AAV Rep 77^{LG}, 79^{FA}, and 192^{HG} modified proteins using the known sequence of AAV2 which includes the AAVRep78 sequence.

Applicant submits that a deposit is not necessary as the nucleic acid and amino acid sequences are disclosed in the specification, in a publication and publicly available online . Applicant requests that the Examiner reconsider his position and withdraw this rejection.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 7, 14, 15, 17and 18

Withdrawn

Claim 7 remains rejected as being indefinite because the Examiner alleges that it is unclear how long the truncated version of the protein is. The Examiner is referred to the specification on page 13, beginning on line 14, where "the smallest truncated AAVRep78 mutant" that is still capable of binding to the target DNA to inhibit the papillomavirus or oncogene as compared to the corresponding wild-type AAVRep 78 protein defines the smallest truncated mutant encompassed by the claim. Applicant submits that the claims define a functional property of the truncated protein and the specification discloses the complete sequence of the AAVRep78 protein. Therefore, the claimed "truncated AAVRep78 protein" is not indefinite to the skilled person because the claim and the specification provides an unambiguous definition of these truncated proteins.

Withdrawn

Claims 14, 15, 17 and 18 are rejected as being indefinite for the lack of disclosure for the specific sequence for the tat protein of HIV and/or not providing a reference a reference of the specific sequence. On page 14, lines 27-28 of the specification, it is disclosed that a publication by Nagahara *et al.* discloses the use of the tat protein in fusion proteins. Also attached is an HIV Sequence Database provided by the U.S. government (Attachment 10) showing tat alignments on line. Additionally, a search of the NCBI Protein sequence database shows almost 1700 hits of the tat protein (Attachment 11). Applicant submits that tat protein of HIV is well known and available to skilled persons, and it is requested that this rejection be withdrawn.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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